Preliminary environmental assessment of Flubendazole use in Scotland for parasitic worm control in Moorland Grouse

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Executive Summary

The 2019 Scottish Government's Grouse Moor Management Group's report ("the Werritty report") included an investigation into the use of medicated grit containing the active ingredient flubendazole for the treatment and prevention of the strongyle worm (*Trichostrongylus tenuis*) in red grouse. Based on concerns that there may be risks for the environment when best practice is not followed, the Werritty report made several recommendations in relation to the use of medicated grit as well as an additional recommendation that "SEPA should initiate a desk-based study to determine the appropriate nature and extent of a monitoring programme to ascertain whether flubendazole residues exist in water bodies on or downstream from where it is being used, including in association with grouse moors, to conduct such a monitoring programme and to report on its findings".
SEPA committed to undertake a desk-based study to assess the potential impact on the wider environment of the use of flubendazole in medicated grit. This report is the result.

The active ingredient (a.i.) flubendazole is added to grit in a stearate coating, with around 50mg a.i. present in a 500g grit tray. Based on typical stocking densities, over a 3000 ha grouse moor there would be a total of around 100g flubendazole used each season following treatment recommendations.

Flubendazole will enter the environment via the faeces of treated grouse and by leaching from spilt or open piles of medicated grit if best practice is not followed. Based on these release pathways and the substance's properties, flubendazole may be found in soil, surface waters and sediments on or near moorlands. No measured environmental data exist in relation to this use of flubendazole.

Exposure in animals that may predate or scavenge on grouse carcasses is likely to be low, as are levels in soil, based on typical dose rates. However, predicted levels in surface water and sediments are less certain because of missing information on the importance of particle-associated transport during runoff following rainfall. It is clear though that levels in watercourses are likely to be higher in cases of malpractice where grit is placed too near a watercourse. These variables also mean that monitoring in practice would not be a good tool to pick up examples of poor practice in a systematic way.

It has not been possible to derive a toxicity threshold for the protection of the water environment because of missing data for some organisms. The available data show that aquatic invertebrates, e.g. water fleas, are more sensitive to the substance than aquatic plants. Comparing the most sensitive aquatic toxicity test result to predicted levels shows that the risk to surface water organisms in watercourses on or near grouse moors is likely to be low when best practice is followed. The biggest areas of uncertainty in this conclusion relate to poor grit placement practice and to a lesser extent inputs associated with particulate runoff. Extrapolating the aquatic toxicity test result to sediments leads to the same conclusion on risk, with similar caveats. Further extrapolating the aquatic toxicity test result to soil-dwelling organisms indicates that the risk again is likely low.

Although the assessment in this report has generally presented a low environmental risk from the use of flubendazole in medicated grit on grouse moors, the uncertainties in both the estimated environmental concentrations and ecotoxicological effects data are high enough for us to recommend:

- An investigation into levels of flubendazole in surface waters (and sediments) and potentially impacted invertebrate communities near or on moorlands using medicated grit be considered to confirm that best practice is protective of the local environment. Sampling for chemical analysis should be conducted to reflect both dry and wet periods and be carried out during the main periods of use (winter – early summer)
- If there is a need to investigate instances of grit malpractice, “walk overs” or other visual inspection methods (eg unmanned aerial reconnaissance) should be considered since ecological and chemical monitoring are unlikely to give much information on this.
- Refining this report should new information on ecotoxicity or modelling approaches relevant for particulate run off become available to improve predictions of levels in water and sediments and their potential impact on wildlife.

Contents

1. Introduction

In 2017 the Scottish Government established the Grouse Moor Management Group to examine the environmental impact of grouse moor management practices. In November 2019 the group submitted its final report[1] ("the Werritty report"; Werritty et al 2019) to the Cabinet Secretary for Environment, Climate Change and Land Reform. The report included an investigation into the use of medicated grit for the treatment and prevention of the strongyle worm (*Trichostrongylus tenuis*) in the gut of Red Grouse. It concluded that "*there is some evidence that prescription levels are too high, that gritting holidays are not always observed, and that grit may not always be withdrawn from grouse at least 28 days before Red Grouse enter the food chain. At present there is little evidence of a resistance problem with the use of medicated grit, but there is some evidence that flubendazole is toxic to aquatic organisms*". The Werritty report made several recommendations in relation to the use of medicated grit, as well as an additional recommendation that "*SEPA should initiate a desk-based study to determine the appropriate nature and extent of a monitoring programme to ascertain whether flubendazole residues exist in water bodies on or downstream from where it is being used, including in association with grouse moors, to conduct such a monitoring programme and to report on its findings*". Following publication of the report and subsequent discussion in the Scottish Parliament, SEPA committed to undertake a desk-based study to assess the potential impact on the wider environment of the use of flubendazole in medicated grit, including an assessment of the effects on soil dwelling organisms, aquatic organisms and the indirect effect on predatory animals (from the consumption of contaminated prey). This report documents this study.

Products containing the active ingredient flubendazole are usually added to feed to control worm infestations in chickens and fowl. The strongyle worm can cause cyclical fluctuations in grouse numbers every 6-9 years in Scotland, and the use of grit coated in a product containing flubendazole has substantially suppressed these cycles since its introduction in 2007. Flubendazole medicated grit for grouse is not officially authorised by the Veterinary Medicines Directorate (VMD). However, use is allowed under the "cascade" process as part of the Veterinary Medicines Regulations 2013. Medicated grit is offered through prescription by a vet and it is recommended follows predetermination of worm levels in grouse. There is currently no overarching system in place that monitors the use of medicated grit. There is no obligation for landowners to follow best practice, as laid out

[1] Available at https://www.gov.scot/groups/grouse-moor-management-group/

in *Moorland Management Best Practice Worm Control in Red Grouse Guidance* (Scotland's Moorland Forum, 2018) and *Best practice use of medicated grit* (GWCT, 2019). This means there are likely to be cases where treatment is offered as a precaution without prior determination of worm burdens, and it is "contested evidence" in the Werritty report that led to the concerns that prompted this report.

The Werritty report found that, when used correctly, the use of flubendazole has been highly effective in reducing endemic strongyle levels in grouse and that residues in grouse for human consumption present a very low risk. There is a trade off between frequency of treatment and breeding success, with "gritting holidays" recommended when worm burdens are low (to lower the risk of drug resistance developing). Treatment must also be ceased 28 days before birds are shot by law. Initiatives such as Scotland's Moorland Form's Worm Control in Red Grouse Guidance and GWCT's Best practice use of medicated grit, plus workshops provided by the Moredun Research Institute, aim to address this at the voluntary level. However, the Werritty report found anecdotal evidence that grit trays were not being used (open piles of grit), including near to watercourses (GWCT recommends trays not be placed near to surface waters, although no minimum distance seems to be recommended) leading to the additional recommendation for SEPA quoted above.

2. Information on the substance

In the compilation of this report the open literature was searched for published papers relating to flubendazole's properties, use, ecotoxicity and presence in the environment. The authors also made contact with researchers working in the area of grouse moor best practices and relevant regulators in relation to authorised uses of products containing flubendazole and any relevant grey literature.

2.1 Intended uses

Flubendazole is one of a family of benzimidazoles used as anthelmintics (treatment of intestinal parasitic worms in livestock and humans). In the UK it is authorised for use in pigs and poultry as a feed or drinking water additive. Although use quantities are not available, the VMD Product Information Database lists eight products that are currently authorised for use in the UK (five for use in pigs, six in chickens and four in geese, partridge, pheasants or turkeys)[2]. Although authorised for use in humans in Europe, it is not listed on the British National Formulary and does not seem to be prescribed or available in over the counter products (NHS Scotland, personal communication).

[2] https://www.vmd.defra.gov.uk/productinformationdatabase/ accessed 3rd September 2020

Medicated grit is available by veterinary prescription only and as flubendazole is not licensed for use in grouse in the UK, prescribing products containing it is achieved through application of the cascade mechanism. Based on low exposure to the environment in its various uses, no higher tier prospective assessment for the environment was carried out as part of the authorisation process (VMD, personal communication). For its use in grouse treatments, flubendazole is the active ingredient in Flubenvet, present at 5% w/w, incorporated into a stearate coating onto grit (for other livestock incorporation into feed is recommended). Dose quantities vary but are around 1kg of the product in 1 tonne of grit (equating to approx. 50g flubendazole per tonne).

2.2 Substance Identification

Flubendazole (CAS 31430-15-6; IUPAC name methyl N-[6-(4-fluorobenzoyl)-1H-benzimidazol-2-yl]carbamate; figure 1) is a substituted benzimidazole anthelmintic that works by binding to β-tubulin and inhibiting microtubule formation in the intestinal cells, inducing decreased glucose uptake and starving of the parasites (Martin 1997). Wagil et al (2015) state that microtubules serve a variety of important functions in animal, plant, fungi and some bacterial cells so evaluating these anthelmintics for potential effects in aquatic flora and fauna is relevant.

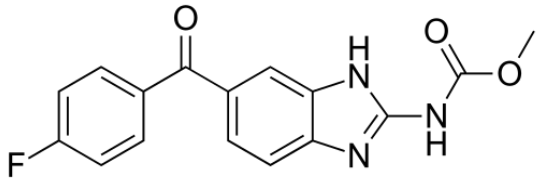

Figure 1: molecular structure of flubendazole

2.3 Physico-chemical properties

Flubendazole has low water solubility (<10 mg/l, likely <0.05mg/l based on analogue data), an appreciable octanol-water partition coefficient (log Kow 2.91) and is ionisable with pKa values of 3.6 and 9.6; at environmentally relevant pHs the substance will almost entirely exist in its neutral form (Wagil et al 2015 and references therein).

2.4 Fate and Behaviour

In soil, the substance is rather immobile with a high measured water-organic carbon partition coefficient (log Koc >3.94 L/kg & 4.00 L/kg (Bundschuh et al 2016)). Half-lives in water and sediment (at 20 deg C) are given as 60 and 542 days respectively (Bundschuh et al 2016). Degradation studies in manure and manured soils also indicate it is relatively persistent in the terrestrial environment (DT50 174 days in clay soils after standard manure application; Kreuzig et al 2007). The substance's lack of primary degradation is likely a result of its poor

bioavailability. There is conflicting evidence as to the substance's photodegradation, but this removal mechanism will be less relevant in top soils and for adsorbed substances. The substance's log Kow indicates it is unlikely to bioaccumulate.

3. Exposure assessment

Flubendazole is present at 5% w/w in the prescribed product, incorporated into a stearate coating onto grit. Dose quantities vary but are around 1kg of the product in 1 tonne of grit (equating to approx. 50g flubendazole per tonne). Grit trays are the preferred method of deployment since these control quantities available and minimise environmental release of uneaten grit. The grit is now stearate coated and so is less likely to allow significant leaching (72% remained on weathered grit after 1 year). The Game and Wildlife Conservation Trust recommend one grit tray per breeding pair, with no more than 500g of grit per tray (GWCT, 2015). As grouse typically consume 35g of grit/month, a tray will last a pair approx. seven months (note this approach differs greatly from other livestock, where treatment in feed lasts only for seven consecutive days). Treatment must be withdrawn at least 28 days before animals are shot for subsequent human consumption. Generally, grit trays are placed at the end of the shooting season in late autumn and are removed one month before the start of the shooting season in early august (GWCT, 2020). The period of potential release is therefore winter, spring and early summer.

The GWCT has a number of best practice guidelines which include a number of measures to reduce potential environmental exposure. These include:

- Conducting early and late autumn counts of strongyle worms from at least 20 shot birds. Where counts are low, consider delaying treatment until counts of worm eggs in grouse faeces are conducted in the winter.
- The use of grit trays as opposed to placing grit in piles on the soil surface.
- Avoid siting trays near standing or running water.
- Supply a maximum of 500g of medicated grit per breeding pair per season.

Toxicokinetic studies in other bird species (chickens and turkeys) indicate that flubendazole is absorbed rapidly but that a high proportion is eliminated unchanged in faeces. Organ specific analysis showed that, of the systemically available flubendazole, this undergoes a high level of metabolism in the liver. For this reason maximum residue limits (MRLs, relevant for human consumption) are based on the parent compound

and a “marker” metabolite (EMEA, 2006). It must be noted that these data are based on in-feed studies; there may be differences in toxicokinetics from ingested grit treatments as well as species-dependent differences.

The primary pathways of exposure in the environment from use in medicated grit will include the soil compartment (mainly from unchanged flubendazole in faeces, and potentially from direct losses to moorland if best practice not followed).

For aquatic exposure, flubendazole’s affinity to sorb to soil and low water solubility means that emissions to groundwater are less likely, and releases to surface water are more likely to result from flubendazole bound to particulate runoff rather than in solution/sub-surface pore water.

3.1. Measured Environmental data

SEPA do not have any aquatic monitoring data for flubendazole. Data from one partner organisation in England indicate very low frequencies of detection at low ng/l levels in surface water, however the location of sites monitored are unlikely to relate to use of the substance in medicated grit on moorlands.

3.2 Predicted Environmental Concentrations for Soil

The Predicted Environmental Concentration for Soil (PEC_{soil}) has been calculated on the basis that the main release pathway is from unmetabolised flubendazole in grouse faeces and takes into account the accumulation of the substance in soil over subsequent years of treatment. The derivation follows the principles of the guidance published as part of the Veterinary International Conference on Harmonisation (VICH) for veterinary medicines registration (European Medicines Agency, 2016), although there is no exposure scenario that directly relates to releases from grouse on moorland. The closest scenario is excretion by livestock on pastureland; this scenario has been adapted to derive a PEC_{soil} in this assessment. The calculations and parameters used to derive PEC_{soil} are presented in Appendix C. Note that while this scenario does not take into account direct leaching of the substance from spilt or open grit mounds, as it assumes all of the grit is used over a season and that all of the dose is excreted by the bird, in terms of mass balance all of the flubendazole is accounted for. The final PEC_{soil} calculated is 1.14 µg/kg.

3.3. Predicted Environmental Concentration for Surface Waters (including sediment)

It is assumed that flubendazole will mainly enter surface waters in dissolved form (from soil porewater) and sorbed to soil particles in rain

run off. Following VICH guidelines a PEC_{water} can be estimated from the concentration of the substance in soil pore water (derived from the soil PEC above) with an assumed dilution factor of 3 for the receiving water body. This gives a PEC_{water} of 0.475 ng/l (see appendix C). This approach does not account for concentrations of the substance sorbed to particulates in runoff, which is likely to be a much higher route of exposure based on the substance's properties (and highly relevant for the sediment compartment).

The substance may also enter watercourses directly from grit trays or piles that are placed too near water courses. This route of exposure would reflect intermittent releases related to periods of heavy rainfall that could potentially result in periodically significant concentrations in water. No model is available to model such inputs, and uncertainties around specifics such as rainfall, surface absorbance, run off rates etc make this scenario difficult to model from first principles. This is further discussed in appendix C, but as a worst case (assuming 10% of offered grit is spilt) up to 1.4mg flubendazole per tray could leach over the course of a treatment season or up to 14mg in the case of a pile of grit (if for some reason the pile remains unused).

A $PEC_{sediment}$ can be calculated from the PEC_{water} above based on the substance's partitioning behaviour (see appendix c). As above, this only considers dissolved losses from soil that then partition to sediment once in the receiving water and not losses of flubendazole attached to particulate runoff, or intermittent direct releases from grit placed near to watercourses. The $PEC_{sediment}$ is 0.238 µg/kg.

4. Effects data

No harmonised EU classification is available for flubendazole. A majority of notifiers to the EU classification database list the substance as toxic for reproduction cat.2 (H361d; Suspected of damaging fertility or the unborn child). There are no notified classifications for the environment[3].

4.1. Aquatic organisms

Although limited, there are data available on the toxicity of flubendazole for invertebrates, aquatic plants and bacteria. Notably, no data could be found for effects on fish. Data are summarised in appendix A. Although a thorough review of reliability of the studies has not been undertaken, all the studies retrieved appear to have been conducted to accepted laboratory and scientific principles.

For acute data, the most sensitive species were invertebrates: flatworm (*Dugesia gonocephala*, 96 hour-EC50 21.9 µg/l, Bundschuh et al.,

[3] https://www.echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database/-/discli/notification-details/123043/796374; accessed 21st August 2020.

2016), tubicified worm (*Tubifex tubifex*, 96hour-EC50 22.1 µg/l, Bundschuh et al., 2016) and water flea (*Daphnia magna,* 48 hour-EC50 55.4 µg/l (mean of four studies; Wagil et al., 2015, Oh et al 2006, Bundschuh et al 2016, & Puckowski et al 2017). Tests on other trophic levels were limited, but no effects on green algae or marine bacteria were found at the maximum concentrations tested (>1000 µg/l and >300 µg/l respectively, Bundschuh et al., 2016).

There are only chronic data available for *Daphnia magna.* The lowest effect concentration was a 21 day NOEC for growth of 2.5 µg/l (Oh et al., 2006).

In the absence of data on the toxicity of flubendazole to fish a predicted no effect concentration (PNEC) has not been derived in this assessment.

4.2. Sediment dwelling organisms

Flubendazole has a log Koc of 4.00 (log Kow 2.91) which, following WFD Common Implementation Strategy Guidance Document 27 (EC, 2018), means sediment dwelling organisms are relevant receptors. The literature search returned no relevant sediment toxicity data, but it is possible to use the equilibrium partitioning approach with the aquatic toxicity data and partitioning data to determine a screening level threshold for sediment toxicity. It should be noted that Koc values are highly dependent on the test system used to generate them, and for this reason amongst others assessments based on equilibrium partitioning are generally used to assess the need for further testing to refine the risk assessment rather than give conclusive information on levels of risk.

Equilibrium partitioning calculations are presented in Appendix B. The lowest chronic aquatic toxicity endpoint of 2.5 µg/l translates to 0.482 mg/kg in sediment.

4.3. Soil dwelling organisms

No data could be found on the impacts of flubendazole on soil dwelling organisms. As for the assessment of sediment dwelling organisms, equilibrium partitioning can be used to estimate toxicity in soil dwelling organisms based on aquatic toxicity data (EC 2008 & EC 2012). The same caveats apply for the approach used for this compartment, with the additional caution that the estimated toxicity level is if anything more uncertain than that for sediment (the REACH guidance from where this approach is taken states that aquatic toxicity may only be applicable to organisms with a water permeable epidermis, and for certain species direct exposure to soil may be of greater importance than exposure via food). Appendix B details the calculation that relates the lowest chronic aquatic toxicity endpoint of 2.5 µg/l to a value of 0.653 mg/kg in soil.

4.4. Higher organisms (Secondary Poisoning)

There are three potential exposure pathways for higher organisms; those that predate on soil dwelling organisms like earthworms or, in the aquatic environment, fish, and scavengers/predators of treated birds.

i) worm-eating predators and fish-eating predators: under the REACH Regulation (EC,2008) and WFD guidance (EC, 2018) a cut off Log Kow of 3 is used to assess the need for a secondary poisoning assessment in the absence of data on bioaccumulation potential. This value is used to indicate that exposure via accumulation in the foodchain is low. Flubendazole has a Log Kow of 2.91 and therefore does not meet the criterion for secondary poisoning assessment.

ii) scavengers/predators that may consume treated birds: exposure via this route is likely to be low because much of the administered dose is excreted in faeces or metabolised in the liver. In addition, flubendazole is of low acute oral toxicity in poultry, rats and dogs (EMEA, 2006). Sub-chronic and chronic studies found equivocal or limited effects for relevant endpoints. The WHO/FAO Joint Expert Committee on Food Additives (JECFA) calculated an average daily intake (ADI) for humans of 0-12 µg/kg bw per day by applying a safety factor of 200 to the NOEL of 2.5 mg/kg bw per day which was established in the 3-month study in dogs (EMEA, 2006).

Hazards to higher organisms are therefore deemed to be low.

In summary, flubendazole is likely to pose the greatest hazard to aquatic and sediment dwelling invertebrates. There may also be a relevant hazard to soil dwelling invertebrates given flubendazole's properties. No relevant terrestrial or sediment-dwellers' ecotoxicity data were found to allow a definitive conclusion on these hazards.

5. Preliminary Risk Assessment

As Predicted No Effect Concentrations (PNECs) have not been derived (see section 4 and appendix B) for soil and water compartments, this section presents the predicted environmental concentrations alongside thresholds of toxic effect to give some context to the sections above. In order to develop risk quotients (ie PEC/PNEC) for the compartments to conclude on levels of risk, as well as more certain PECs additional ecotoxicity data would be needed to derive PNECs.

5.1. Surface waters

Comparing the PECs derived in section 3.3 with levels of effect in aquatic organisms may underestimate the level of risk for the water compartment because two potentially significant exposure routes are not included in the PEC calculation. Based on the release of the substance

in its dissolved form to a watercourse alone the measured no observed effect threshold in the water flea is about 5000 times higher than the water concentration. For sediment, with the same caveats as for the watercolumn plus uncertainty in the effects threshold, the estimated no observed effect threshold is about 2000 times higher than the sediment concentration.

5.2. Soil

The estimated threshold for effects in soil dwelling organisms that is derived from aquatic data (see section 4.2) is around 500 times higher than the PEC_{soil} (see section 3.2 and appendix C). Whilst there is uncertainty in the values, the PEC_{soil} is based on birds excreting all of the flubendazole they consume unmetabolised and accounts for year on year use and release. So this comparison can be considered to represent a reasonable worst case.

6. Conclusions and recommendations

Information on environmental exposure of flubendazole for its use in medicated grit on moorlands for the treatment of worm in grouse is lacking. The most relevant pathways to the environment are via the faeces of treated birds and leaching from spilt or open piles of medicated grit, if best practice is not followed. Based on these pathways of release and the substance's properties, the target environmental compartments are soil and surface waters (the latter likely to be from both dissolved fraction and sorbed particulate run off).

Estimates of environmental exposure on and near moorlands are uncertain. However, based on some conservative assumptions levels in soil are likely to be low. Concentrations dissolved in surface waters are also likely to be low, but it has not been possible to model concentrations associated with particulate runoff. This is likely to be a more significant transport pathway than the dissolved fraction given flubendazole's properties.

Once in water, flubendazole is likely to partition to sediment. Sediment exposure has been estimated based on the dissolved fraction entering watercourses only. This means water and sediment concentrations may be underestimated especially in the case of malpractice (i.e. spilt or open piles of medicated grit placed too near watercourses). This is a datagap in the exposure assessment that would require further work.

As the daily dose in the grouse is low, the substance has a low potential for bioaccumulation and the majority of the administered dose that is absorbed is metabolised. Exposure in animals that may predate or scavenge on grouse carcasses is therefore likely to be low.

Ecotoxicity data for flubendazole are limited to marine bacteria, aquatic plants and invertebrates. Invertebrates are more sensitive to the substance than the other tested species. These data have been used to estimate equivalent levels of toxicity for soil- and sediment-dwelling organisms. Estimates, however, are uncertain and can only be used to form indicative conclusions on hazard for these environmental compartments. Taking account of these uncertainties predicted thresholds of effect are orders of magnitude above compartment specific predicted concentrations as modelled in this report.

Although the assessment in this report has generally presented a low environmental risk from the use of flubendazole in medicated grit on grouse moors, the uncertainties in both the estimated environmental concentrations and ecotoxicological effects data are high enough for us to recommend:

- An investigation into levels of flubendazole in surface waters (and sediments) and potentially impacted invertebrate communities near or on moorlands using medicated grit be considered to confirm that best practice is protective of the local environment. Sampling for chemical analysis should be conducted to reflect both dry and wet periods and be carried out during the main periods of use (winter – early summer)
- If there is a need to investigate instances of grit malpractice, “walk overs” or other visual inspection methods (eg unmanned aerial reconnaissance) should be considered since ecological and chemical monitoring are unlikely to give much information on this.
- Refining this report should new information on ecotoxicity or modelling approaches relevant for particulate run off become available to improve predictions of levels in water and sediments and their potential impact on wildlife.

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anthelmintic drugs (fenbendazole and flubendazole) to aquatic organisms" Environ Sci Pollut Res. DOI 10.1007/s11356-014-3497-0.

Appendix A – Summary of flubendazole effects data

Duration	Endpoint	Effect	Species scientific name	Species common name	Effect concentration (mg/l)	Details	Comment	Reference
		Acute data						
		Bacteria						
30 min	EC50	Luminescence inhibition	*V.fischeri*	Marine bacteria	>0.3	Measured concentrations	Study conducted to generally accepted procedures	Wagil et al (2015)
		Primary producers						
48 h	EC50	Growth rate	*L.minor*	Duckweed	>1	Literature method. endpoint based on frond area. Measured concentrations	Study conducted to generally accepted procedures	Wagil et al (2015)
24 h	EC50	Growth	*S.vacuolatus*	Green algae	>1	Literature method Measured concentrations	follows principles of OECD 201 (compara	Wagil et al (2015)

							ble sensitivity to 72 h test).	
Invertebrates								
48 h	EC50	Immobility	*Daphnia magna*	Water flea	0.0665		Study details not retrieved	Oh et al (2006)
48 h	EC50	Mortality	*Daphnia magna*	Water flea	0.0701	OECD 202. Measured concentrations	Study conducted to accepted method	Bundschuh et al (2016)
48 h	NOEC	Mortality	*Daphnia magna*	Water flea	0.0074	OECD 202, measured concentrations	Study conducted to accepted method	Bundschuh et al (2016)
48 h	EC50	Immobility	*Daphnia magna*	Water flea	0.045	OECD 202. Measured concentrations	Study conducted to accepted method	Wagil et al (2015)
48 h	EC50	Immobility	*Daphnia magna*	Water flea	0.0448	OECD 202. Measured concentrations	Study conducted to	Puckowski et al (2017)

							accepted method	
96 h	EC50	Mortality	*Asellus aquaticus*	Aquatic sowbug	>1	Measured concentrations	Study conducted to generally accepted procedures	Bundschuh et al (2016)
96 h	EC50	Mortality	*Amphinemura sulcicollis*	Stone fly	>1	Sediment free study (gauze mesh included).	Study conducted to generally accepted procedures	Bundschuh et al (2016)
24 h	EC50	Mortality	*Caenorhabditis elegans*	Nemotode	>1	Measured concentrations	Study conducted to generally accepted procedures	Bundschuh et al (2016)
24 h	EC50	Mortality	*Brachionus calyciflorus*	Rotifer	>8	Measured concentrations	Study conducted to generally accepted	Bundschuh et al (2016)

							procedures	
96 h	EC50	Mortality	*Dugesia gonocephala*	Flatworm	0.0219	Measured concentrations	Study conducted to generally accepted procedures	Bundschuh et al (2016)
96 h	EC50	Mortality	*Tubifex tubifex*	Tubicified worm	0.0221	Sediment free test (quartz sand only). Measured concentrations	Study conducted to generally accepted procedures	Bundschuh et al (2016)
		Chronic data						
		Invertebrates						
21 days	NOEC	Growth	*Daphnia magna*	Water flea	0.0025		Study details not retrieved	Oh et al (2006)
25 days	NOEC	Growth	*Daphnia magna*	Water flea	0.00625		Study details not retrieved	Oh et al (2006)

21 days	NOEC	Reproduction	*Daphnia magna*	Water flea	0.01	Study details not retrieved	Oh et al (2006)
25 days	NOEC	Reproduction	*Daphnia magna*	Water flea	0.025	Study details not retrieved	Oh et al (2006)

Appendix B – Equilibrium partitioning for sediment & soil toxicity assessment

The approach to equilibrium partitioning (EqP) is taken from the European Commission Common Implementation Strategy 27 on deriving EQS (EC, 2018) and underlying REACH guidance (EC, 2008).

1) Sediment

As there are not enough data to calculate a water PNEC, the lowest available pelagic chronic endpoint has been used instead as an indicator of sediment toxicity. This assessment assumes that sediment-dwelling organisms are of similar sensitivity to pelagic organisms, that sediment pore water concentrations are related to water column concentrations (ie partitioning processes are at equilibrium), and that exposure occurs mainly via the pore water (for substances with log Kow <5).

The partition coefficient between solid-water in sediment is calculated by:

$$Kp_{sed} = Foc_{sed} \times K_{oc}$$

Where Kp_{sed} is the partition coefficient between solid-water in sediment ($m^3\ m^{-3}$), Foc_{sed} is the weight fraction of organic carbon in sediment (kg kg^{-1}) set as default 0.05 and K_{oc} is the partition coefficient between organic carbon and water (L kg^{-1}), where for flubendazole a value of 10,000 (log Koc 4) L kg^{-1} has been used. The Kp_{sed} for flubendazole is therefore:

$$0.05 \times 10000 = 500$$

This value is used to derive the partition coefficient between sediment and water as:

$$K_{sed-water} = Fair_{sed} \times K_{air-water} + Fwater_{sed} + Fsolid_{sed} \times \frac{Kp_{sed}}{1000} \times RHOsolid$$

Where, $K_{sed-water}$ is the partition coefficient between sediment and water, $Fair_{sed}$ is the fraction of air in sediment ($m^3\ m^{-3}$) set at default 0, $K_{air-water}$ is the air-water partition coefficient ($m^3\ m^{-3}$), $Fwater_{sed}$ is the fraction of water in sediment ($m^3\ m^{-3}$) set at default 0.8, $Fsolid_{sed}$ is the fraction of solids in sediment ($m^3\ m^{-3}$) set at 0.2 and $RHOsolid$ is the density of the solid phase ($kg_{solid}\ m_{solid}^{-3}$) set at default of 2500. $K_{air-water}$ is calculated using the equation below:

$$K_{air-water} = \frac{H}{R \times TEMP}$$

Where, H is the Henry's law constant (Pa m^3 mol^{-1}) which is 2.73 x 10^{-13} (based on EPIsuite prediction) for flubendazole, R is the gas constant (Pa m^3 mol^{-1} K^{-1}) set at default 8.314 and TEMP is the environmental temperature (K) set at 285. $K_{air-water}$ for flubendazole is therefore:

$$\frac{2.73 \times 10^{-13}}{8.314 \times 285} = 8.98 \times 10^{-17}$$

The $K_{sed-water}$ can therefore be calculated as:

$$0 \times 8.98 \times 10^{-17} + 0.8 + 0.2 \times \frac{500}{1000} \times 2500 = 250.8 \text{ m}^3 \text{ m}^{-3}$$

The sediment water coefficient ($K_{sed-water}$) can then be used to determine the wet weight equilibrium partitioning quality standard for sediment ($QS_{sediment,EqP,ww}$) as:

$$QS_{sediment,EqP,ww} = \frac{K_{sed-water}}{RHO_{sed}} \times QS_{fw,eco} \times 1000$$

Where, $QS_{sediment,EqP,ww}$ is the wet weight equilibrium partitioning quality standard for sediment, RHO_{sed} is the bulk density of wet sediment which is set as default 1300 kg_{ww} m^{-3} and $QS_{fw,eco}$ is the freshwater quality standard. In this case there is no quality standard or PNEC derived for water, so the lowest chronic endpoint (0.0025 mg L^{-1}) has been used instead to give an indication of possible toxicity to sediment dwelling organisms calculated in the same way:

$$\frac{250.8}{1300} \times 0.0025 \times 1000 = 0.482\, mg.kg^{-1}$$

2) Soil

The same process can be used to estimate toxicity to soil dwelling organisms following guidance developed for the REACH Regulation (EC, 2008). The same principles and caveats apply, although less validation work has been undertaken for the application of EqP for the soil compartment and there are indications that the approach is more uncertain for the soil compartment than sediment.

The partition coefficient between solids and water in soil is calculated by:

$$Kp_{soil} = Foc_{soil} \times K_{oc}$$

Where Kp_{soil} is the partition coefficient between solids and water in soil ($m^3\ m^{-3}$), Foc_{soil} is the weight fraction of organic carbon in soil ($kg\ kg^{-1}$) set as default 0.02 and K_{oc} is the partition coefficient between organic carbon and water ($L\ kg^{-1}$), where for flubendazole a value of 10,000 (log Koc 4) $L\ kg^{-1}$ has been used. The Kp_{soil} for flubendazole is therefore:

$$0.02 \times 10000 = 200$$

This value is used to derive the partition coefficient between soil and water as:

$$K_{soil-water} = Fair_{soil} \times K_{air-water} + Fwater_{soil} + Fsolid_{soil} \times \frac{Kp_{soil}}{1000} \times RHOsolid$$

Where, $K_{soil-water}$ is the partition coefficient between soil and water, $Fair_{soil}$ is the fraction of air in soil ($m^3\ m^{-3}$) set at default 0.2, $K_{air-water}$ is the air-water partition coefficient ($m^3\ m^{-3}$), $Fwater_{soil}$ is the fraction of water in soil ($m^3\ m^{-3}$) set at default 0.2, $Fsolid_{soil}$ is the fraction of solids in soil ($m^3\ m^{-3}$) set at 0.6 and $RHOsolid$ is the density of the solid phase ($kg_{solid}\ m_{solid}^{-3}$) set at default of 2500. $K_{air-water}$ is calculated using the equation below:

$$K_{air-water} = \frac{H}{R \times TEMP}$$

Where, H is the Henry's law constant ($Pa\ m^3\ mol^{-1}$) which is 2.73×10^{-13} (based on EPIsuite prediction) for flubendazole, R is the gas constant ($Pa\ m^3\ mol^{-1}\ K^{-1}$) set at default 8.314 and TEMP is the environmental temperature (K) set at 285. $K_{air-water}$ for flubendazole is therefore:

$$\frac{2.73 \times 10^{-13}}{8.314 \times 285} = 8.98 \times 10^{-17}$$

The $K_{soil-water}$ can therefore be calculated as:

$$0.2 \times 8.98 \times 10^{-17} + 0.2 + 0.6 \times \frac{200}{1000} \times 2500 = 300.2\ m^3\ m^{-3}$$

The soil water coefficient ($K_{soil-water}$) can then be used to determine the wet weight equilibrium partitioning quality standard for soil ($QS_{soil,EqP,ww}$) as:

$$QS_{soil,EqP,ww} = \frac{K_{soil-water}}{RHO_{soil}} \times QS_{fw,eco} \times 1000$$

Where, $QS_{soil,EqP,ww}$ is the wet weight equilibrium partitioning quality standard for soil, RHO_{soil} is the bulk density of wet soil which is set as default 1150 kg_{ww} m^{-3} and $QS_{fw,eco}$ is the freshwater quality standard. In this case there is no quality standard or PNEC derived for water, so the lowest chronic endpoint (0.0025 mg L^{-1}) has been used instead to give an indication of possible toxicity to soil dwelling organisms, but calculated in the same way:

$$\frac{300.2}{1150} \times 0.0025 \times 1000 = 0.653\, mg.kg^{-1}$$

Appendix C – Predicted Environmental Concentrations

1) PEC Soil

The PEC soil calculations are based on the VICH guidelines (EMEA, 2016). There are no exposure scenarios that exactly reflect excretion of flubendazole by grouse on moorland. The closest is excretion by livestock on pasture. The calculations associated with this scenario have been used to calculate a PEC_{soil} for flubendazole excretion by grouse.

The first calculation is to calculate $PEC_{soilinitial}$ which is the initial predicted environmental concentration of flubendazole in soil and is derived using:

$$PEC_{soil\ initial} = \left(\frac{D \times Ad \times BW \times SD \times Fh}{1500 \times 10000 \times 0.05}\right) \times 1000$$

Where, $PEC_{soil\ initial}$ is the initial predicted environmental concentration of flubendazole in soil (µg/kg), D is the daily dose of the active ingredient (mg/kg_{bw} per day) which has been calculated as 0.3735 mg/kg_{bw} per day based on an assumption of 500g of grit dosed with 50 mg of flubendazole treating two birds per season at a mean grouse weight of 600g. Ad is the number of days of treatment (d) assumed to be 100 days based on GWCT (2020), BW is the animal body weight (kg_{bw} per animal) assumed to be 0.6 (Wildlife Trust, 2020), SD is the stocking density (animal/ha) set as 1.43 grouse per hectare based on mean grouse density data presented in Werrity et al (2019), Fh is the fraction of the animals treated, it is set as 1 assuming all grouse receive an equal dose. The numbers 1500, 10000 and 0.05 relate to the bulk density of dry soil (kg/m^3), area of 1 hectare (m^2) and depth of soil penetrated (m) respectively. The number 1000 is a conversion factor so that the final units are in µg/kg.

The $PEC_{soil\ initial}$ is therefore calculated as:

$$PEC_{soil\ initial} = \left(\frac{0.3735 \times 100 \times 0.6 \times 1.43 \times 1}{1500 \times 10000 \times 0.05}\right) \times 1000$$

$$PEC_{soil\ initial} = 0.0427\ \mu g/kg$$

This calculation does not account for year on year accumulation of flubendazole in soil. This is a relevant consideration as flubendazole has a DT_{50} in clay soil of 174 days (Kreuzig et al., 2007). The PEC_{soil} can be further refined to account for this using the following equation:

$$PEC_{soil\ 1\ year} = PEC_{soil\ initial} \times e^{\left(\frac{(-\ln 2 \times 365)}{DT_{50}}\right)}$$

Where $PEC_{soil\ 1\ year}$ is the PEC in the soil 1 year after the start of treatment, and DT_{50} is the soil half life (days). $PEC_{soil\ 1\ year}$ is therefore calculated as:

$$PEC_{soil\ 1\ year} = 0.0427 \times e^{\left(\frac{(-\ln 2 \times 365)}{174}\right)}$$

$PEC_{soil\ 1\ year} = 0.0411\ \mu g/kg$

The fraction of flubendazole degraded one year after application is calculated as:

$$Fs = \frac{\left(PEC_{soil\ initial} - PEC_{soil\ 1\ year}\right)}{PEC_{soil\ initial}}$$

$$Fs = 0.0374$$

The $PEC_{soil\ plateau}$ is the PEC in soil at plateau (µg/kg) and is calculated as:

$$PEC_{soil\ plateau} = \frac{PEC_{soil\ initial}}{Fs}$$

$$PEC_{soil\ plateau} = 1.14\ \mu g/kg$$

The plateau PEC_{soil} (ie approximates to steady state) estimated in the assessment is therefore 1.14 µg/kg.

2) PEC surface water

In the VICH guidelines PEC aquatic is calculated by determining the concentration of the substance in soil pore water and assuming a

dilution by the receiving water body. This approach does not account for losses of the substance attached to particulates in runoff. To do this a separate assessment is required.

To calculate PEC aquatic the initial PEC soil is converted to a wet weight PEC and to a soil depth of 20cm. For this the $PEC_{soil\ plateau}$ will be used:

$$PECsoil_{plateau\_pw\_dw} = \frac{PEC_{soil\ plateau}}{4}$$

$$PECsoil_{plateau\_pw\_ww} = \frac{PECsoil_{pleateau\_pw\_dw}}{CONV_{soil}}$$

Where, $PECsoil_{plateau\_pw\_ww}$ is the PEC soil corrected to wet weight and soil depth of 20 cm (µg/kg) and $CONV_{soil}$ is the dry to weight wet conversion factors set as 1.13 kg_{dw}/kg_{ww}. $PECsoil_{plateau\_pw\_ww}$ is therefore 0.252 µg/kg.

$PEC_{porewater\ is}$ calculated using the following equation:

$$PEC_{porewater} = \frac{PECsoil_{plateau\_pw\_ww} \times RHO_{soil}}{K_{soil-water} \times 1000}$$

Where, RHO_{soil} is the bulk density of fresh soil set as 1700 kg/m^3 and $K_{soil-water}$ is the partition coefficient between solids and water in soil which was calculated as 300.2 m^3/m^3 in appendix B.

$$PEC_{porewater} = \frac{0.252 \times 1700}{300.2 \times 1000}$$

$$PEC_{porewater} = 0.00142\ \mu g/l$$

To derive PEC surface water, the PEC porewater is simply divided by 3 to account for dilution in the receiving water body. PEC surface water is therefore 0.000475 µg/l.

However, this **does not** account for flubendazole sorbed to solid particles in runoff.

Release to the water environment is also possible in wet conditions direct from medicated grit that has spilt from grit trays or been left in open piles when they have been positioned near to watercourses. No modelling scenario was found for this exposure route and owing to the high number of site-specific variables, attempts to estimate release rates and a PEC_{water} for this scenario have not been successful. To give some context to the potential release based on each grit tray containing 500g

of grit dosed with 50mg flubendazole at the start of the gritting season, and assuming around 10% of the grit is spilt and around 28% of the dose leaches over the course of a year's weathering, over that period 1.4mg of flubendazole could be released per tray. For open grit piles quantities leached would be higher. As a worst case, if a grit pile remained unused for some reason, up to 14mg flubendazole could leach from it over the gritting season. As one grit tray (or pile) is supplied for every 1.4 ha (based on mean grouse density data and 1 pair of birds using one tray/pile as presented in Werrity et al 2019) and moor sizes can vary between 200 and 10,000 ha, this scenario could be important and requires further investigation either through modelling or monitoring.

3) PEC sediment

PEC sediment is calculated using the VICH guidelines. However, like the PEC surface water calculation it only considers losses in solution that then partition into sediment once in the receiving water and not losses of flubendazole attached to particulate runoff that then settle as fresh sediment. It is derived using the following equation:

$$PEC_{sediment} = \frac{K_{sed-water}}{RHO_{sed}} \times PEC_{surface\ water} \times 1000 \times CONV_{sed}$$

Where, $K_{sed-water}$ is the sediment-water partition coefficient which was calculated as 250.8 m^3/m^3 in appendix B, RHO_{sed} is the bulk density of sediment set at 1300 kg_{wwt}/m^3 and $CONV_{sed}$ is the conversion factor for sediment from wet weight ti dry weight, set at 2.6.

$$PEC_{sediment} = \frac{250.8}{1300} \times 0.000475 \times 1000 \times 2.6$$

$$PEC_{sediment} = 0.238\ \mu g/kg$$

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